

# Floral color shift and entomophily in the hermaphroditic weed, *Phyla nodiflora* (L.) Greene (Verbenaceae)

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## ABSTRACT

*Phyla nodiflora* is a widespread perennial weed and sexually active throughout the year. The flowers are hermaphroditic, weakly protandrous and facultative autogamous involving self-pollination by pollen fall on the stigma by gravity and self- and cross-pollination by insects. They are white with yellow corolla throat, nectariferous on the day of anthesis and meant for pollination by autogamy and insects. They are light pink with dark purple throat on subsequent days, nectarless and meant for long distance attraction for pollinating insects, increased pollen deposition and seed set. Seed dispersal modes include autochory, hydrochory, zoochory and anthrophochory. Asexual mode is also functional. Hermaphroditism, facultative autogamy, self-pollination with or without insects, cross-pollination by insects, floral color shift, different seed dispersal modes, sexual and asexual modes collectively empower the plant to grow as a widespread weed. It can be controlled especially in agricultural areas by promoting its use in traditional medicine and as a pioneer species in eco-restoration areas.

**Keywords:** *Phyla nodiflora*, hermaphroditic, facultative autogamy, floral color shift, entomophily, seed dispersal modes.

## 1. INTRODUCTION

The genus *Phyla* is from the Greek word "phyle" for clan or tribe, alluding to the many flowers per spike while *nodiflora* refers to flowers from the nodes. This genus is characterized by its prostrate herbaceous plants rooting at the nodes, malpighiaceae hairs and uncinata hairs on the calyx. With these characters, it is distinguished from *Lippia* genus which is characterized by its spreading shrubs with simple, non-malpighiaceae hairs and uncinata hairs lacking on the calyx (Greene 1899). *Phyla* is a small genus in the tribe Lantaneae of the family Verbenaceae and represents five species and three varieties of which four are found only in the Americas and one species, *P. nodiflora* is widespread in tropical and temperate areas of the world. In *P. nodiflora*, three varieties have been distinguished on the basis of morphological characters, var. *nodiflora*, var. *minor* and var. *reptans*, of which the first one is distributed worldwide while the other two are distributed in certain tropical and temperate areas of the world (O'Leary and Mulgura 2012). Later, Weakly

(2015) distinguished var. *nodiflora* and var. *minor* based on leaf characters; leaves are obovate and spatulate with obtuse apex and sub-glabrous texture or with scattered sub-appressed hairs in var. *nodiflora* while they are elliptic or obovate shape with an acute apex and a densely white-strigose or canescent texture in var. *minor*. Gross et al. (2017) mentioned that the classification of varieties in *P. nodiflora* has not been done based on molecular study. Verdecourt (1992) documented that *P. nodiflora* occurs in various habitats. Sharma and Singh (2013) reported that *P. nodiflora* is distributed in India, Sri Lanka, Ceylon, Baluchistan, South and Central America, and Tropical Africa. It is used for pain relief in knee joints, for regulation of bowel movement, treatment of ulcers and boils, swollen cervical glands and gonorrhea.

Estes and Brown (1973) reported that *P. incisa* requires probing into the corolla tube by insects for autogamous reproduction. Gori (1983) mentioned that *P. incisa* changes color after pollination. Gross et al. (2010) *P. canescens* is native to South America but grows as a weed in many regions of the world. It is self-compatible but not capable of automatic self-pollination and hence is obligately vector-dependent for pollination to produce seed set; *Apis mellifera* is the principal pollinator. Gross et al. (2017) noted that *P. nodiflora* is capable of producing seed through autonomous autogamy. Deyrup and Deyrup (2015) reported that *P. nodiflora* is visited by *Apis mellifera*, *Augochloropsis metallica*, *Halictus poeyi*, *Lasioglossum lepidii*, *Anthidium maculifrons*, *Coelioxys mexicana*, *C. texana*, *Megachile brevis pseudobrevis*, *M. mendica* (bees), *Ammophila urnaria*, *Bicyrtes insidiatrix* and *Prionyx thomae* (wasps). Despite the widespread distribution of *P. nodiflora* in the tropical and temperate world and its value in traditional medicine, it has not been investigated for its pollination ecology. Therefore, the present study is an attempt to provide certain details of pollination ecology of *P. nodiflora* var. *nodiflora* in order to understand its ability to succeed as a perennial mat-forming weed in diverse habitats across tropical and temperate areas of the world.

## 2. MATERIALS AND METHODS

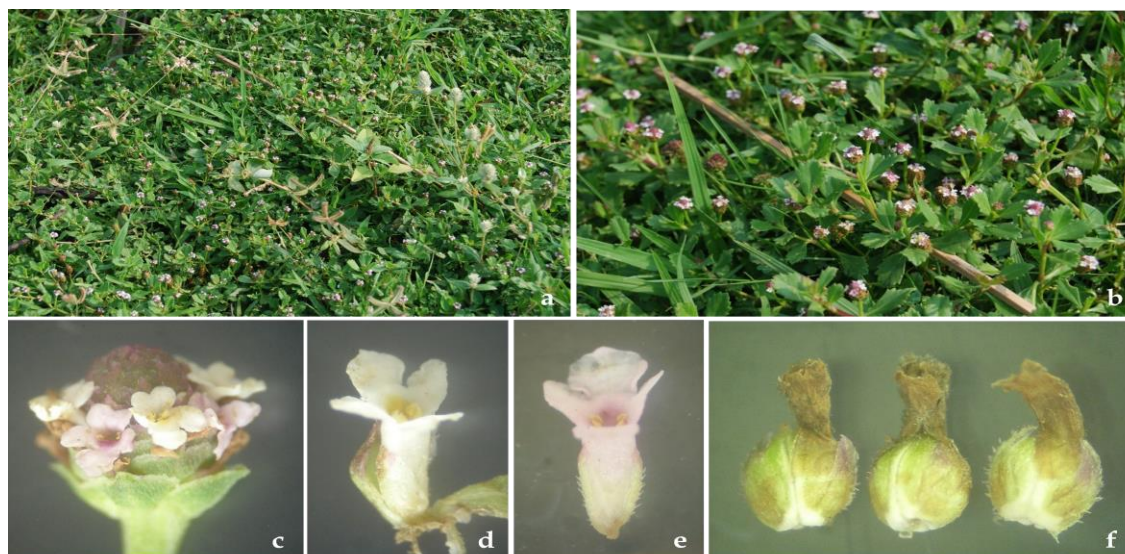
*Phyla nodiflora* growing in open damp sites of Andhra University campus was used for study during July to December 2019. In this study, *P. nodiflora* phenology, flowering season, floral biology, foraging activity and pollination, fruiting and seed dispersal aspects were investigated in the field. Flower visitors were observed from morning to evening identify species and their foraging behavior for the collection of nectar and/or nectar. Stigma receptivity was tested according to the protocol described in Dafni et al. (2005). Twenty inflorescences were bagged prior to the initiation of flower production to test autogamy and another set of thirty inflorescences were tagged prior to the initiation of flower production in open-pollinations. These two modes of pollination were followed for 3 weeks to record fruit and seed set rates separately. Fruiting behavior, seed dispersal modes and seed germination were observed in the field to evaluate the ability of the plant to grow as a widespread weed.

## 3. RESULTS

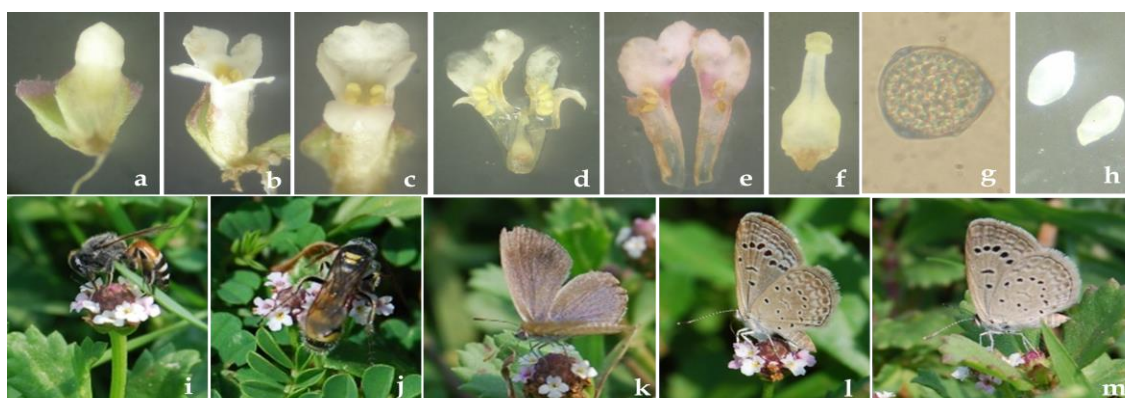
**Phenology:** It is a small prostrate perennial mat-forming marshy herb with woody rootstock and roots at distal nodes (Figure 1a). The rooted portion of nodes subsequently gets detached and forms a new plant. It grows in open and wet places near streams, ponds, agricultural fields along bunds and irrigation canals. The stem is much branched, slender and green to purplish in color. Leaves are petiolate, born in pairs at stem nodes, fleshy, obovate-spathulate, base cuneate, apex obtuse and margins sharply serrate with scattered and sub-appressed hairs; these leaf characters indicate that the plant is var. *nodiflora* according to Weakly (2015). The plant exhibits prolific vegetative growth and profuse flowering and fruiting during rainy season while sparse vegetative growth and sporadic flowering and fruiting during late winter and dry season (Figure 1b). At population level, the plant displays vegetative growth, flowering and fruiting alternately or simultaneously in different habitats. With perennial habit, it creeps along the ground forming roots as it spreads and also sprouts back to life from woody rootstock.

**Flower morphology and floral biology:** Inflorescence is a pedunculate and bracteate, cylindrical, 1.5-2.5 cm long, purplish head consisting of tiny clustered zygomorphic hermaphroditic flowers and usually borne at alternate nodes and rarely in both axils at the same node. The flower head is placed above the leaves very prominently by its elongate peduncle and produces flowers acropetally for about one week (Figure 1c). The buds gradually mature and open daily at 0700-0800 h (Figure 2a,b). The flowers are tubular at their base with a 2-lipped cupular calyx, almost equal to the length of the corolla tube; the lobes are lanceolate and surrounded by longer broad overlapping bracts. The corolla is 2-lipped with 2-lobed upper lip and 3-lobed lower lip, salver-form, 2-2.5 mm long, glabrous; the corolla tube is 1.5-2 mm long. Initially, the corolla is white with yellow center (Figure 1d) and gradually towards the evening turns to light pink with dark purple throat (Figure 1e). The stamens are 4, didynamous, included, united to the middle portion of the corolla tube at two levels and placed at the throat of the corolla (Figure 2c-e); the anthers are dithecous, light yellow, basifixed and dehisce by longitudinal slits. The pollen grains are spheroidal, tricolporate, sub-psilate, 24.75 µm on polar axis and

31.5  $\mu\text{m}$  on equatorial axis (Figure 2g). The pistil consists of mono-carpellate ovary with two 1-ovuled locules, short style and obliquely capitate stigma (Figure 2f,h).



**Figure 1.** *Phyla nodiflora*: a. Habit, b. Flowering phase, c. Acropetal anthesis, d. White flowers with yellow corolla throat, e. light pink flowers with dark purple throat, f. Fruits.



**Figure 2.** *Phyla nodiflora*: a. Mature bud, b. Flower, c-e. Placement of stamens at corolla throat in white and light pink flowers, f. Pistil, g. Pollen grain, h. Ovules, i. *Apis florea*, j. *Campsomeriella* sp., k. *Freyeria trochylus*, l. *Zizeeria karsandra*, m. *Zizina otis*.

**Pollination:** The flowers are nectariferous with traces of nectar secreted at the base of the corolla, weakly protandrous with anthers dehiscing during mature bud stage and the stigma attaining receptivity during anthesis and extending its receptivity until the evening of the day of anthesis. The stigma is placed below the lower pair of stamens and is self-pollinated by pollen from the anthers of the same flower falling down by gravity but this mode of pollination occurs only when pollen is dry which occurs from late morning to late evening. The stigma is either self or cross-pollinated throughout the day by insect activity. The insect visitors included *Apis cerana*, *A. florea* (Figure 2i) (Apidae bees), *Campsomeriella* sp., (Figure 2j) (Scoliidae wasp), *Chilades pandava*, *C. laius*, *Freyeria trochylus* (Figure 2k, *Zizeeria karsandra* (Figure 2l) and *Zizina otis* (Figure 2m) (Lycaenidae butterflies). All these insects were regular and consistent foragers and their activity was very intense in damp sites where the plant produced mat-like populations.

The inflorescences display two floral colors, white and light pink. The white flowers are fresh and nectariferous with yellow corolla throat which serves as nectar guide to direct the probing insects towards nectar location. Insects visited these flowers legitimately by probing through the corolla throat to access pollen and nectar and effected either self or cross-pollination. The white flowers begin to show corolla color change from the late evening of the day of anthesis; the whole corolla turns light pink with dark purple throat on the 2nd day and the light pink flowers usually lack nectar, and remain in place even after fruit formation. The insect foragers initially probed light pink flowers at the beginning of the day and as the day progressed, they totally avoided visiting these flowers and concentrated only on white flowers with yellow corolla throat. The discrimination of floral colors by



insects indicated that white flowers are rewarding and meant for pollination while light pink flowers are non-rewarding and meant for long distance attraction for pollinating insects.

**Fruiting and seed dispersal:** Fruits mature within 2-3 weeks depending on the moisture and nutrient environment of the soil. They are enclosed by persistent calyx until they are mature and dry, and also by the corolla but the latter gradually withers and wilts. Fruit set was 89% in bagged flowers and 94% in open-pollinations. Seed set was 67% in bagged flowers and 85% in open-pollinations. Fruit is a hard compressed oblong schizocarp consisting of 2 yellowish tan mericarps which separate upon maturity. (Figure 1f). Each mericarp is ovoid, rounded on one side and flattened on the other side with one tiny brown oval and flattened seeds; it separates at maturity exposing the seeds. Seed dispersal is autochorous; they are initially dispersed below the parent plants and subsequently dispersed by rain water, animals and humans. Seeds germinate immediately and form new plants in wet habitats while they show delayed germination in semi-dry habitats to form new plants. As a perennial herb, the plant produces several generations in a year in damp sites.

#### 4. DISCUSSION

*P. nodiflora* is a widespread weed in tropical and temperate areas of the world (O'Leary and Mulgura 2012). Two varieties, *nodiflora* and *minor* in *P. nodiflora* have been distinguished based on leaf characters by Weakly (2015). Variety *nodiflora* is characterized by obovate and spatulate leaves with obtuse apex and sub-glabrous texture or with scattered sub-appressed hairs while variety *minor* is characterized by elliptic or obovate shaped leaves with an acute apex and a densely white-strigose or canescent texture. In the present study, *P. nodiflora* leaf characters indicate that it represents var. *nodiflora*. Verdecourt (1992) documented that *P. nodiflora* grows in diverse habitats, in coastal dune systems, along sandy lake shores, shore muds, grasslands, roadsides and salt marshes. In the study area, it grows in diverse habitats representing wet and moist soil environment and forms huge populations carpeting the ground. The crowded form of floral display is quite distinct because of the placement of cone-shaped inflorescence on an elaborate peduncle above the leaves and such floral display facilitates long distance attraction to pollinating insects.

Weiss and Lamont (1997) reported that insect-pollinated plants show floral color change which occurs in the whole or certain localized parts of flowers. The color change occurs simultaneously in flowers that change whole. The color change in localized parts of flowers is more common among insect-pollinated species but moth-pollinated flowers show whole flower color change only. Insect pollinators concentrate their foraging visits only on fresh rewarding flowers (Gori 1983; Lamont 1985; Weiss 1995). Both plants and insects benefit from this interaction, the former receives efficient pollination service while the latter are accurately directed to rewarding flowers. Cruzan et al. (1988) reported that *Phyla incisa* flowers show two phases, younger, nectar containing white flowers with yellow corolla throat and older, nectar-lacking flowers with dark purple corolla throat. These authors documented that bees and bee-flies preferred to visit yellow-throated flowers while they rarely visited the purple-throated flowers; butterflies were much less selective in discriminating one of these floral phases. The older-phase flowers lack pollinator resources and pollinators may learn to discriminate them to concentrate on flowers that provide pollinator resources. But, older-phase flowers increase the visitation rate of pollinators to inflorescences and as a result, the pollen removal rate increases with little effect on pollen deposition on stigmas. *P. nodiflora* in the study area also shows two floral color phases, fresh white nectar containing flowers with yellow corolla throat and mostly nectar lacking older, light pink flowers with dark purple throat. Bees and wasp species commonly foraged on white flowers but these insects also made rare attempts to probe light pink purple flowers initially but having learned that they do not have pollinator resources, they subsequently discriminated and concentrated only on white flowers for pollinator resources which include pollen and nectar for bees and only nectar for the wasp. However, nectar-seeking lycaenid butterflies are not very selective in choosing white flowers as bees and the wasp did indicating that lycaenids do not have any discriminatory power to distinguish between rewarding and non-rewarding flowers. The inflorescence top serves as a landing place for flower-probing insects and several small white flowers with upwardly placed corolla throats produced around this landing place facilitate the insects to probe flowers easily. The change in the entire corolla color and loss of stigma receptivity by the evening of the first day of anthesis indicate that light pink flowers do not require pollination but they function for long distance attraction of pollinators. Further, color difference in the corolla between rewarding and non-rewarding flowers allow pollinating bees and the wasp to forage relatively efficiently upon their landing on the inflorescence. Further, the retention of light pink flowers in the inflorescence may also contribute to the success of sexual reproduction through increased pollen donation when pollinator activity is high and increase the probability of seed set even when pollinator activity is low or pollinators are rare. Similar situation has been reported in *P. incisa* by Cruzan et al. (1988). It is also pertinent to mention that change in flower color serves as a cue to searching pollinators informing them of non-availability of pollinator resources in flowers that display shift in corolla color and this cue is used by the pollinators to avoid non-rewarding flowers and increase their search for rewarding flowers (Gori 1983). The pollen

grains with distinct exine ornamentation and other characters constitute a particular category of pollen and this pollen type is reported *Phylla nodiflora*-type by Perveen and Qaiser (2007). The characters of these pollen grains appear to be adapted for adhering to the specific parts of the probing bees and the wasp and to the proboscis of probing lycaenid butterflies in order to be efficiently transported for increasing pollination rate.

Estes and Brown (1973) reported that *P. incisa* requires probing by insects for autogamous seed production. Gross et al. (2010) reported that *P. canescens* does not produce seed through autogamy. Gross et al. (2017) mentioned that *P. nodiflora* is capable of autogamous seed production as 83% of flowers bagged by them produced seed automatically. In this study, fruit and seed set rates of *P. nodiflora* in bagged and open-pollinations indicated that it is capable of autogamous and allogamous but highest fruit/seed production is a function of insect-mediated self and/or cross-pollination. Therefore, facultative autogamous breeding system is functional in *P. nodiflora*.

Different authors reported on the seed dispersal of *P. nodiflora*. Pettingill (1939) and Rundle and Sayre (1983) mentioned that *Phylla* seeds have been found in the stomachs of migratory birds in North America and hence seed dispersal in *P. nodiflora* by water birds is one possibility. Razi (1950) reported the *P. nodiflora* seeds disperse by endo- and ectozoic means in India. Dostine and Morton (2000) reported that *P. nodiflora* seeds have been found in the stomachs of the Comb Crested Jacana (*Irediparra gallinacea*) distributed in the northern Australia. It is a water bird common to Asia and Australia that is dispersive among water bodies. Baldwin et al. (1996) noted that sea currents may be a pathway for the pantropical distribution of *P. nodiflora* as its seeds are tolerant to inundation and saline conditions, and as it grows well in coastal sand dunes in Africa (Abuodha et al. 2003) and in Australia (see Gross et al. 2017). Further, seed dispersal in *P. nodiflora* is also human-mediated (Gross et al. 2017). In this study, *P. nodiflora* has been found to be autochorous as mericarps of the schizocarpic fruit separate at maturity to facilitate self-seed dispersal. It is also hydrochorous, zoochorous and anthropochorous. Seeds germinate immediately and produce several generations in a year if the soil environment is favorable. Further, the plant has the ability to sprout back to life from the woody rootstock and produce new plants from the rooting ability of creeping stems at node points. Therefore, the sexual and asexual modes of reproduction and different modes of seed dispersal empower the plant to grow as a successful weed and become widespread in diverse habitats of the world.

Sharma and Singh (2013) reported that *P. nodiflora* is used for pain relief in knee joints, for regulation of bowel movement, treatment of ulcers and boils, swollen cervical glands and gonorrhea. Since this weed is widespread both in agricultural and non-agricultural areas, it can be controlled by promoting its use in traditional medicine. Its ability to grow close to the ground carpeting the soil enables it to provide excellent ground cover. Its ability to grow in sunlit and shade areas, adapt to drought conditions and withstand short period of flooding or standing water makes it a versatile pioneer species for use in the restoration of ecologically degraded, damaged and destroyed habitats/ecosystems.

## 5. CONCLUSION

*P. nodiflora* is a widespread perennial weed and sexually active throughout the year. The cone-shaped inflorescence with a display of several small white and light pink flowers in a circle elevated on an elaborate peduncle serves as a long distance attractant to pollinator insects. The flowers are hermaphroditic, weakly protandrous and facultative autogamous involving self-pollination by pollen fall on the stigma by gravity and self- and cross-pollination by insects. They are white with yellow corolla throat on the day of anthesis and light pink on subsequent days. The white flowers are nectariferous and meant for pollination by autogamy and insects while light pink flowers are nectarless and meant for long distance attraction for pollinating insects, increased pollen donation when pollinator activity is high and increased probability of seed set when pollinator activity is low or pollinators are rare. Seed dispersal modes include autochory, hydrochory, zoochory and anthropochory. Asexual mode is also functional. Hermaphroditism, facultative autogamy, self-pollination with or without insects, cross-pollination by insects, floral color shift, different seed dispersal modes, sexual and asexual modes collectively empower the plant to grow as a widespread weed. It can be controlled especially in agricultural areas by promoting its use in traditional medicine and as a pioneer species in eco-restoration areas.

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### Authors' contributions

All three authors contributed equally.

**Conflict of Interest**

The authors declare that there are no conflicts of interests.

**Funding:**

This study has not received any external funding.

**Ethical approval**

The ethical guidelines for plants & plant materials are followed in the study for species collection & identification.

**Data and materials availability**

All data associated with this study are present in the paper.

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